

Application No. 10/081,885
Amendment dated November 3, 2003
Reply to Office Action of July 1, 2003

Amendments to the Specification:

Please replace the paragraph beginning at page 7, line 9, with the following amended paragraph:

In a method for diagnosing SPMD in an individual, first a sample of muscle tissue from the individual is provided and, if necessary, treating to render the components of the tissue available for antibody binding, the muscle tissue sample being characterized by levels of the $\alpha 7\text{A}$ integrin protein; contacting the muscle tissue sample with an antibody which specifically binds to the $\alpha 7\text{A}$ integrin protein, wherein said contacting under conditions appropriate for binding of the antibody to the $\alpha 7\text{A}$ integrin protein; detecting the extent of binding of the antibody to the $\alpha 7\text{A}$ integrin protein in the muscle tissue sample; and comparing the extent of binding of the antibody specific for the $\alpha 7\text{A}$ integrin protein in the muscle tissue sample from the individual for whom diagnosis is sought to the extent of binding of the antibody specific for the $\alpha 7\text{A}$ integrin protein in a muscle tissue sample from a normal individual, wherein a substantial reduction in the extent of binding of the antibody specific for the $\alpha 7\text{A}$ integrin protein in the muscle tissue sample from the individual for whom diagnosis is sought as compared with the extent of binding in the muscle tissue sample of a normal individual is indicative of SPMD (scapuloperoneal muscular dystrophy). Desirably the muscle tissue samples are from skeletal muscle tissue. Histological specimens from an individual for whom diagnosis is sought and from a normal individual can also be used with antibody detection methods. Detection of the bound antibody can be via a detectable label such as a fluorescent compound, a chemiluminescent compound, radioactive label, enzyme label or other label known to the art, coupled with detection methods obvious in choice to one of ordinary skill in the art. A second antibody which recognizes the (first) integrin-specific antibody can be labeled and used to detect the bound first antibody. Advantageously, assays can be run in parallel for the assessment of the expression of 2/4 laminin

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in the individual for whom diagnosis is sought (and in a normal (control) sample. In an SPMD patient, the laminin levels are within the normal range.

Replace the paragraph beginning at page 11, line 3, with the following amended paragraph:

Mutations in the $\alpha 7$ integrin gene resulting in the absence or reduction of the $\alpha 7$ integrin protein have been shown to be responsible for the myopathy and delayed motor milestones of ~~4~~ 3 Japanese patients with previously undefined muscular dystrophies (Hayashi et al., 1998). In addition, expression of the $\alpha 7\beta 1$ integrin protein has been shown to be up-regulated in Duchenne muscular dystrophies (DMD) and down-regulated in laminin-2/4 ($\alpha 2\beta 1\gamma 1$)-deficient patients. Because of the role of the $\alpha 7\beta 1$ integrin in muscle development, structure and function, we have further examined of its involvement in human muscle disease. Laminin-2/4 is also known as merosin. The structural gene encoding the $\alpha 7$ integrin has been mapped by fluorescence in situ hybridization (FISH) and radiation hybrid mapping to human chromosome 12q13. Given the genetic localization of the SPMD to 12q13-15 and the role of the $\alpha 7$ protein in muscle development and function, we have concluded that lack of expression of this gene is the underlying cause of this progressive muscle wasting disease.

Replace the paragraph beginning at page 21, line 6, with the following amended paragraph:

To determine if the $\alpha 7$ integrin polypeptide is involved in SPMD, muscle biopsies taken from five patients with SPMD were analyzed for integrin expression. Using immunofluorescence and western blot analyses analyses, it was shown that there was a marked reduction or absence of the $\alpha 7\beta 1$ integrin in all five SPMD patients as compared with normal healthy controls. In contrast,

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the $\alpha 7\beta$ integrin was detected in the lining of the blood vessels, suggesting that aberrant tissue specific gene expression or alternative RNA splicing may cause the lack of this integrin in skeletal muscle. Immunofluorescence analysis revealed an increase in levels of dystrophin in muscle fibers of SPMD patient tissue samples; perhaps dystrophin compensates for the reduced integrin linkage system in skeletal muscle. In addition, utrophin expression, normally confined to neuromuscular junctions, was observed throughout the muscle membranes of SPMD patients. Our results indicate that the reduction (or lack) of $\alpha 7\beta 1$ integrin in skeletal muscle contributes to SPMD.

Delete line 4 at page 27:

Example 6. Immunofluorescence Analyses:

Replace the paragraph beginning at page 28, line 8, with the following amended paragraph:

The primers used to amplify around the human $\alpha 7A/\alpha 7B$ human $\alpha 7A\alpha 7B$ alternative splice site are hu3101F 5'-GAACAGCACCTTCTGGAGG-3' (SEQ ID NO:4) and hu3438R 5'-CCTTGAAGTGTGTCGGTCT-3' (SEQ ID NO:5). In SPMD patients there is very little $\alpha 7A$ amplification product in comparison to the amount seen in a normal individual. The expected product sizes from the use of these primers in a polymerase chain reaction are for $\alpha 7A$: 451 bp band; $\alpha 7B$: 338 bp band. The numbers in the primer names correspond to the location in the human cDNA sequence, F denotes a forward primer and R denotes a reverse primer.